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Quantitation of the Immunocompetence of the Dental Pulp

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QUANTITATION OF THE IMMUNOCOMPETENCE
OF THE DENTAL PULP

BY

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A Thesis Submitted to the Faculty of the Graduate School
Of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

JUNE

1974

DEDICATION

To my wife, Patricia, my love, who
lends her strengths so together
we can live life to its fullest.

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I would like to express my deep appreciation to the following people.

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CHAPTER I

INTRODUCTION

Almost every clinical discipline in medicine has been influenced by the rapid advances of immunology in the past ten years. In dentistry, the field of oral pathology and periodontics have also undergone many important advances. It is time therefore that certain aspects of pulp biology and endodontics be considered in the light of immunobiology. The state of our scientific knowledge concerning inflammation of the dental pulp is at best empirical. Research shows most inflammation of the dental pulp is due to microorganisms or their metabolites, but no one has ever correlated this knowledge with the classical immunological concepts of inflammation. It is my contention that pulpal biology and pulpal inflammation should be examined in this manner. In order to begin, one must study the normal and compare it to the abnormal. It is the purpose of this study to select the pulps of normal healthy teeth and quantitate the major components of their immunologic system, IgG, IgA, IgM, and component C'3 of complement. Taking these results I will address myself to unhealthy teeth with inflamed pulps and compare the values obtained.

CHAPTER II

REVIEW OF THE LITERATURE

A. Antibodies: Structure and Function

Immunoglobulins are protein molecules which function as specific antibodies and effect the humoral aspects of immunity. These proteins share many antigenic, structural, and biological similarities, but differ in primary amino acid sequence permitting their antibody function to be highly specific. The history of gamma globulins is relatively short, since Tiselius defined them with electrophoresis as recently as 1937. Just as electrophoresis served initially to distinguish gamma globulins from other serum proteins, additional biochemical and immunochemical techniques made it clear that the gamma globulins were not a single group of proteins. Deutsch, et al (1946), showed most gamma globulins to have a molecular weight of about 160,000. Muller-Eberhard (1956), however, did ultracentrifugal studies which revealed approximately 10% of the gamma globulins in man are gamma macroglobulins, having a molecular weight of about 1,000,000 and a sedimentation rate of around 18s. At the other end of the scale, the Bence-Jones proteins

were found to be microglobulins with molecular weights of about 20,000 - 50,000 and sediment coefficients of 2-4 (Svedberg and Pedersen, 1940; Rundles, et al, 1951; ten Thije, 1956; and Putnam and Miyake, 1957). In 1963, Fahey and Lawrence, separated the serum proteins by electrophoresis. The major fractions found were albumin and gamma globulins. The gamma globulins were further separated into a group of antigenically related proteins composed of three major groups: 6.6.s gamma globulin which comprises 71% of the total gamma globulin. β_{2A} globulin which comprises 22% of the gamma globulins and γ_{1M} macroglobulins comprising approximately 7% of the total gamma globulin protein. The term gamma globulins is commonly used in a somewhat broader context to describe those serum proteins with antibody characteristics, which include the gamma globulins, but extend into the beta and alpha 2 range of electrophoretic mobility (Figure 1). The description and comparison of such immunoglobulins has been facilitated by the adoption of a unified nomenclature summarized in Table 1.

While studying rabbit immunoglobulins, Porter (1959) found that the gamma globulin molecule was too large to determine its function and relate function to its structure. He then carried out experiments degrading the molecule with crystal papain. This technique yielded three fragments

Figure 1
Electrophoretic Pattern
of Serum Immunoglobulins

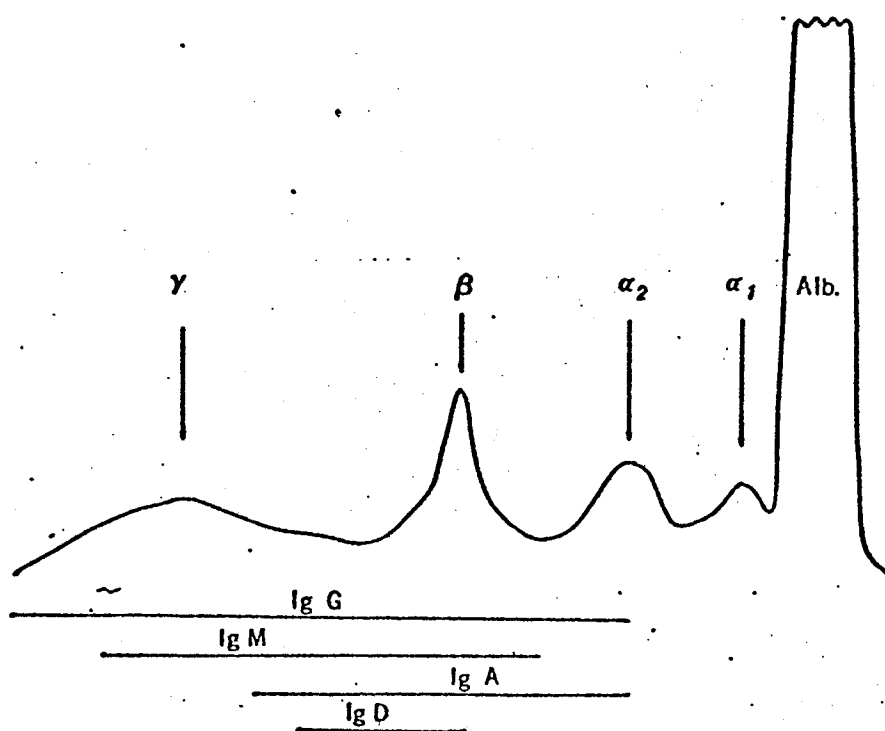


TABLE I
Nomenclature of Immunoglobulins and Their Subunits

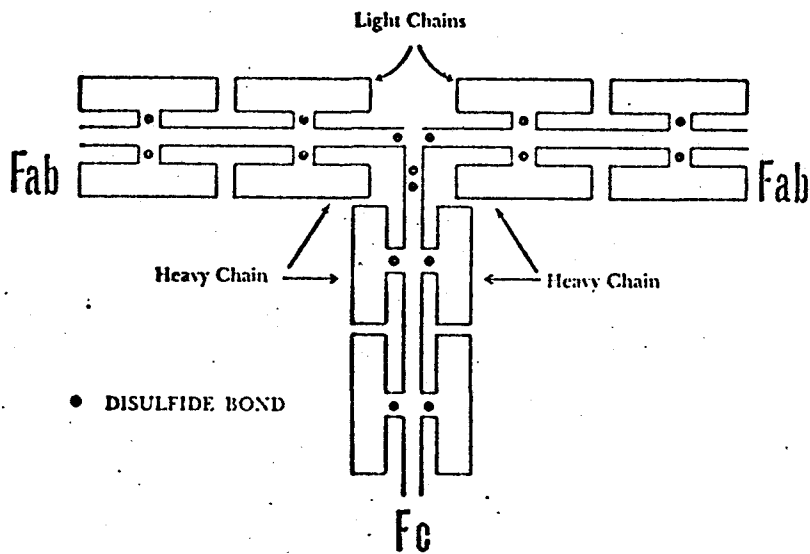
Substance	Recommended Nomenclature	Previous nomenclature
Immunoglobulins	IgG or γ G IgA or γ A IgM or γ M	γ^2 , γ ss, 7SY, 6.6 SY γ^1 A, β^2 A γ^1 M, β^2 M, 19 γ γ -macroglobulin
Papain fragments	IgD or γ D Fab Fc Fd F(ab') ² Fab'	I, II, A, C, S, III, B, F, A piece 5 S divalent fragment Univalent fragment
Chains	Heavy chain Light chain	H, A L, BB
Classes of heavy chain		
IgG	γ Chain	
IgA	α Chain	
IgM	μ Chain	
IgD	d Chain	
Types of light chain	K or κ chains L or λ chains	I, B II, A

Adapted from, Clinical Immunology, 1971.

representing over 90% of the molecular weight. Fragments 1 and 2 (subsequently called Fab) had a molecular weight of about 50,000 and retained the capacity to combine with antigens. Fragment 3 (subsequently called Fc had a molecular weight of approximately 80,000 and retained antigenic specificity. These facts lead him to believe that rabbit gamma globulin was composed of three fragments, two similar and one unique. Later studies elaborated in various texts in the field (Clinical Immunology, Progress in Immunology), give further information concerning the papain fragments. The Fc fragment is a dimer of the C-terminal side of the heavy chain, and it contains most of the carbohydrate of the molecule but has no capacity to combine with antigen. Notwithstanding, this fragment does have important biologic functions: it gives the antibody molecule the capacity to fix complement, traverse the placenta or adhere to tissue mast cells. In addition, the Fab fragments retain antibody combining sites thus giving one molecule of antibody two combining sites.

Further investigation in 1962 led Porter to propose a 4-chain polypeptide structure for antibody molecules. He found the molecule to consist of 2 heavy and 2 light chains covalently linked by interchain disulfide bonds. (Figure 2).

Figure 2
Porter Model of IgG



Schematic drawing of γ G globulin in T-shaped model. Each peptide chain is drawn as a continuous line, and disulfide bonds are represented by •. Where intrachain bonds exist, the chain is drawn to form a loop. Extensive overall similarity is apparent in the placement of these loops between light and heavy chains and between the two portions of the heavy chain (adapted from Bellanti, 1972).

Fleischman, Porter, and Press (1963) confirmed the four chain arrangement with biochemical studies of the peptide chains in gamma globulins. This structure has been found to be applicable to all vertebrates having recognizable humoral antibody.

B. The Biological Properties of Immunoglobulins

The biological properties of the immunoglobulins can be grouped into two categories: antigen binding and biological functioning. Combination with the antigen is specific each molecule of antibody binding only to that particular specific antigen molecule for which it was produced. The other biological properties are independent of specificity and are common to all molecules of a given class of immunoglobulins. (See Table 2).

In humans there are at least five major classes of immunoglobulins. These are, in order of description, IgG, IgA, IgM, IgD, and IgE.

IgG has been defined in man as the one class constituting the major proportion of serum immunoglobulins, immunochemically different from the other classes of immunoglobulins (Bernier, 1970; Bull. W.H.O., 1970). According to Barth (1964), IgG achieves a significant concentration in both vascular (approximately 40% total

TABLE II

Some Physical and Biologic Properties of
Human Immunoglobulin Classes

Class	Mean Serum Concen- tration (mg/100 ml)	Molecular Weight	S 20,w	Mean Survival T/2 (days)	Biologic Function
γG or IgG	1240	150,000	7	23	1. Fix complement 2. Cross placenta 3. Heterocytotropic antibody
γA or IgA	280	170,000	7,10,14	6	1. Secretory antibody
γM or IgM	120	890,000	19	5	1. Fix complement 2. Efficient agglutination
γD or IgD	3	150,000	7	2.8	?
γE or IgE	.03	196,000	8	1.5	1. Reaginic antibody 2. Homocytotropic antibody

Adapted from Bellanti, 1972.

IgG), and extravascular (approximately 60% total IgG) fluids. He also demonstrated that this class of globulins has a relatively long half-life, approximating 23 days. As discussed by Bernier (1970), IgG molecules have the physiological ability to cross the placenta, thus aiding immunologic competence in the newborn infant. Further, this class is considered to provide the bulk of immunity in most infective diseases which feature blood born dissemination, including bacteria, viruses, parasites, and fungi.

IgA, originally called Beta-2A, was first described in human serum by Heremans (1959). He found this protein fraction to represent about one-sixth of the total immunoglobulins. Reported concentration levels vary somewhat throughout the literature however, a recent study by Vaerman (1970), using the Mancini method (Mancini et al., 1965), indicated the mean serum level to be 2.63 mg/ml.

In 1963, Tomasi and Zigelbaum, found that IgA represented the main immunoglobulin in human exocrine secretions. Further examination also found concentrations of IgA in human milk, saliva, and tears, as well as fluids of the clostrum, and the respiratory, urinary, and gastrointestinal tracts. Later studies by Tomasi and Bienenstock (1968), showed IgA to have its highest concentration in the

clostrum. Further, plasma cells in the intracellular spaces and blood vessels in and around the lining of secretory tracts seemed to be the main producers of IgA. These findings led Tomasi to conclusion that IgA plays an important part in the regulation of the bacterial and viral flora of mucous membranes. Although human IgA possesses the same fundamental four-chain structure that is found in all immunoglobulins, it is unique with respect to the fact that its heavy chains tend to polymerize. This is especially noticable in IgA obtained from external secretions, which regularly occurs as a dimer (Tomasi et al., 1965).

Historically IgM immunoglobulins were first recognized in the sera of cattle (Hridelberger and Pederson, 1937). Closer examination in 1956 by Muller-Eberhard using ultracentrifugation revealed that human serum had approximately 10% of its gamma globulin fraction composed of gamma macroglobulins with a molecular weight of around 1,000,000. Studies by Barth and associates (1964) showed IgM, due to its large size, is found mostly in the intervascular spaces and cannot pass the placenta. In 1967, Ishizaka and his colleagues, studied IgM and revealed many interesting properties. Due to its size IgM is a good agglutinator of particulate antigens, such as red blood cells or bacteria.

IgM also has the ability to fix complement but has a very low concentration in secretions. Structural studies carried out by Parkhouse, et al., (1970) demonstrated a pentamer shaped molecule made up of subunits remarkably similar to the four-chain structure described for IgG. This molecular structure undoubtedly accounts for the physical properties related above.

IgD is a class of immunoglobulins which have been largely unexplored and as yet IgD has not been assigned a specific biological role. Rowe and Fahey (1965) in a description of a new class of immunoglobulins determined that increased concentrations of IgD are associated with chronic infections. Further examination of this class by Gleich (1969) showed IgD to have antibody activity but he could not define any specific properties.

IgE represents a minor but distinct class of immunoglobulins present in the serum of man and higher primates. Concentration studies carried out by Johansson in 1967, and 1968 show healthy individuals to have mean concentrations below 1 mg/ml. However, IgE concentration can become elevated 2-30 times normal in various diseases, among which the atopic disorders and parasitic infestations appear to be most prominent (Johansson 1967; Hogarth-Scott et al., 1969). Another important property associated

with IgE is its cytotropic ability to bind mast cells and platelets as well as its ability to attach to homologous skin. Thereby being closely associated with the anaphylactic reaction (Bellanti, 1972).

The function of an antibody is to combine with an antigen under physiological conditions. As noted previously the antibody combining sites are found on the Fab fragment but both the heavy and light chains of this molecule are required for optimum antigenic reaction. Recent experimental observations have clearly shown that antibody specificity is determined by the distinctive amino acid sequence of the immunoglobulin polypeptide chains (Cohen and Milstein, 1967). Once an antibody has captured and combined with antigen, the immune response has begun. The immunological responses are classically divided into those mediated by humoral antibodies and those mediated by cells. In either case the most important aspect of the immune response is specificity. The characteristic of this specificity is the ability of the organism to recognize an antigen as foreign and to respond to it by the synthesis of specific reactive immunoglobins, mainly humoral antibodies such as IgG or IgA, and/or the production of specifically synthesized lymphocytes in the case of cell mediated immunity.

C. Complement

In light of what has been discussed about antibodies, it is necessary to include a description of another component of the immune system: complement. The term complement refers to a complex group of enzymes in normal blood serum that working together with antibodies play an important role as mediators of both immune and allergic reactions. Since the reactions in which complement are involved occur in blood serum or in other body fluids these are considered humoral reactions.

As discussed in Frank's review article (1969), the discovery of complement came in the late 1800's. Its presence was noted during studies of the capacity of blood serum to kill certain microorganisms. Antibodies had been discovered a short time earlier, but it was found that their capacity to kill bacteria depended upon the action of another constituent of serum namely alexin, or complement. The terms were intended to indicate that the agent helps the antibody perform its defensive function.

Although the term complement at first referred to an auxiliary factor in serum, it is now known that in reality it is a complex enzyme system composed of nine functional entities comprised of eleven discrete proteins which together represent about 10% of the globulin fraction of

human serum (Muller-Eberhard, 1969). The nomenclature of complement was formulated by members of a complement workshop held in LaJolla, California in 1966. (Muller-Eberhard, 1968). The individual complement components are designated by numbers and the entire system is represented sequentially in the following manner: C'1, C'4, C'2, C'3, C'5, C'6, C'7, C'8, C'9.

The complement sequence of reaction is normally triggered by the combination of an antibody and antigen. Humphrey and Dourmashkin (1965) concluded that two or three, or perhaps only one, IgM molecule attached to a receptor on a sheep red cell could bring about lysis of the cell through complement activation, but that two adjacent molecules of IgG were necessary. They calculated that if the number of antigen sites on a sheep RBC was 600,000 and if IgG molecules attached at random, then approximately 1000 molecules of IgG would be necessary to give, by chance, the occupation of two adjacent sites resulting in an activation of the complement system.

Normal IgG will more readily activate complement if heated to 63°C for 20 minutes. This process aggregates the IgG molecules and presumably is responsible for the activation of complement which has been observed when RBC's are exposed to serum at low ionic strength (Rapp and Borsos,

1963; Mollison and Polley, 1964).

By aggregating different fragments of IgG it has been shown that the complement activation site is located on the Fc portion of the molecule (Muller-Eberhard, 1968).

As was discussed earlier, not all immunoglobulins have the ability to fix complement. In the human system, IgG and IgM seem to have this ability with IgM molecules being far superior due to their pentameric shape (Ishizaka, et al, 1967).

Some of the most important biological reactions associated with the complement system are the: (1) direct killing of cells, (2) enhanced phagocytosis of cells, (3) liberation of vasoactive substances.

D. Direct Killing of Cells

Green, Barrow, and Goldberg (1959) found that cells which have been acted upon by antibody and complement became permeable to small molecules, and they concluded that functional holes had been formed in the cell membrane. Electron-microscopy carried out by Dourmashkin, et al (1962), demonstrated the presence of these holes. Furthermore, treatment of sheep RBC's with Forssman antibody and guinea-pig complement produced holes with a diameter of 8 to 10 nm.

Experiments described by Mollison (1969) showed the

production of holes in the cell membrane leads to loss of cellular osmotic control and subsequent rupture of the cell itself.

E. The Enhancement of Phagocytosis

Johnston, et al (1969), did experiments on enhancement of bacterial phagocytosis by serum. Using serum deficient in specific complement components and by sequential fixation of purified human C'1, C'4, C'2, and C'3, it was shown that these four components, but not the latter-acting components, are necessary for optimal phagocytosis of pneumococci. This finding was similar to experimental data published by investigators for other bacteria (Stiffel et al, 1964; Glynn and Medhurst, 1967). Further studies by Cooper and Becker (1967) described how phagocytosis promoted by whole serum could be inhibited by the aromatic amino acid - containing peptides, glycyl-Tyrosine and glycyl-Leucyl-Tyrosine. From these findings they concluded that peptidase activity of C'3 fixed to an immune complex is essential for phagocytosis.

F. Release of Vasoactive Substances

The activation of complement may produce anaphylatoxin which in turn liberates vasoactive substances from various cells. It is now known that the mediators of anaphylatoxin activity are substances with a molecular weight of approx-

imately 10,000 which are split off from C'3 and C'5 (Cochrane and Muller-Eberhard, 1968). These substances liberate histamine from mast cells and, in rabbits, have been shown to liberate 5-hydroxytryptamine from platelets as well (Humphrey and Jaques, 1955).

In incompatible blood transfusions in man, it is well known that symptoms such as a flushing of the face, and a constricting pain in the chest, as well as other phenomena are encountered only when the antibodies concerned are anti-A ^{BY} ~~on~~ anti-B, which are powerful complement activators (Mollison, 1969). It has been suggested that the liberation of vasoactive substance, during the complement reaction, may cause a slowing of the local circulation and help to isolate antigen-antibody complexes. (Mollison, 1969).

G. Inflammatory Response

Inflammation can be considered as a complex series of events which develops when the body is injured either by a physical or chemical agent, or by an autoimmune process. While there is a tendency in clinical medicine to consider the inflammatory response exclusively in terms of reactions harmful to the body, a more balanced view is that inflammation is essentially a protective response in which the body attempts either to return itself to the preinjury condition or to repair itself after the inflicted injury. The

inflammatory response is an essential protective and restorative function of the body as it attempts to maintain hemostasis in a changing and often adverse environment.

H. Mediators

For the past two decades, knowledge of acute inflammation has slowly increased. In the 1950's, Spector and Willoughby investigated the apparent chemical mediators of the inflammatory process. These included histamine and 5-hydroxytryptamine. Their studies showed 5-hydroxytryptamine to increase capillary permeability in the rat by approximately 100 times that of histamine. Furthermore, both chemicals were found to illicit pain upon injection, leading them to believe that these compounds could be responsible for the pain associated with inflammation.

Since 1965, there has been a new wave of interest concerning the acute inflammatory response. For example, Houck, et al, in 1966, demonstrated that after tissue injury there is a modification of dermal collagenases, which lead to the formation of modified tissue proteins that can activate the complement system. Therefore, the initial mechanism for acute inflammation is thought to be the production of modified proteins, followed by

the activation of the complement system, which leads to the release of vasoactive substances. In experimental models of acute inflammation constructed by Willoughby, et al, in 1960, it was shown that total depletion of hemolytic complement results in a depression of the acute inflammatory response. The involvement of complement in reactions other than immunologically mediated inflammation has been confirmed by Hill and Ward (1971). They demonstrated that split fragments of C'3 are involved in experimental myocardial infarcts in rats. Further studies involving the effect of complement on inflammation show that activation of the complement system leads to the release of a number of vasoactive and phlogistic substances (eg. anaphylatoxin) by the third and fifth components of complement, which cause the release of histamine C567, a chemotactic polymorphonuclear substance (Cochrane and Muller-Eberhard, 1967).

After release of vasoactive substances, the coagulation system as described by Jancso, 1961, is activated. It is Jancso's opinion that the increased vascular permeability allows a coagulation process to occur on the terminal vessel walls (margination). This further enhances vessel permeability and edema formation. Recently, Jancso's hypotheses has been confirmed by experiments

carried out by Ward (1967). He demonstrated one of the clotting components, plasmin, yielding a split fragment of C'3 initiating chemotactic activity.

More recently discovered mediators of inflammation are the prostaglandins. These are substances which are found during the latter stages of inflammation (Willis, 1969, a and b). Giroud and Willoughby (1970) described these substances to be formed partly by the action of complement; in as much as their liberation depends on an intact complement system.

Experimentation with many nonsteroidal anti-inflammatory drugs shows inhibition of the release of the prostaglandins in vitro. (Vane 1971). Further studies by Smith and Willis (1971) showed aspirin therapy selectively inhibits prostaglandin production in human subject's platelets. DiRosa, Papadimitriou, and Willoughby (1972), have shown that the release of prostaglandins is closely associated with the migration of mononuclear cells into inflamed sites. They showed also that non-steroidal anti-inflammatory drugs prevent phagocytosis and leukocyte migration in vitro.

A traditional description of inflammation is given by Florey in his General Pathology text (1970). Generally three states of inflammation can be identified. These

include, acute, subacute, and chronic, each has a typical histological description. The acute inflammatory response begins with the dilation of blood vessels and the effusion of leukocytes and fluids. The result of these processes leads to a loss of the normal capacity of blood vessels to retain fluids and cells, arriving at an edemic condition. Leukocytes are attracted by a localized synthesis of a chemotactic substance, while vascular permeability is altered by such mediators as histamine and 5-hydroxytryptamine.

Within an hour of injury, polymorphonuclear leukocytes (PMN's) make their appearance. They can first be seen clustering along endothelial cells of vessels in the area of injury. This accumulation of cells along the vessel wall is called margination. Soon after this accumulation, these cells migrate and form the first line of defense against infection. Four or five hours later the mononuclear cells (lymphocytes and monocytes) appear, thus bringing more phagocytes (monocytes) and immunologic capacity via the lymphocytes to the area. Up to this point this description covers the protective aspects of acute inflammation, however, an uncontrolled inflammation can lead to severe abscess formations such as those described by Selzer and Bender in the text The Dental Pulp

(1965). As they see it, an outpouring of too much fluid can build up sufficient intrapulpal pressure to cause irreversible damage to the connective tissue. Likewise, the arrival of excessive numbers of PMN's and the subsequent discharge of their proteolytic enzymes

Compromising problems such as these cause the tissue to pass through the subacute phase, which is an accumulation of lymphocytes and monocytes with formation of granulation tissue, into the chronic stage. Chronic inflammation is characterized by the continued presence of mononuclear cells with the appearance of plasma cells. The reason for a progression to this stage rather than a resolution of the inflammation is due to a persistence of foreign material, either viable or necrotic. Persistence of these inflammatory cells can lead to severe functional impairment of a tissue either by immunologically mediated tissue injury or by a cumulating total fibrosis.

In Bellanti's (1971) opinion, tissue injury due to immunologic reactions have their origin in the inflammatory response which is initiated by the reaction to antigens in tissue. The initiating event may be either a mediator such as the leukotactic factor generated from the complement system or a factor produced by the invading

microorganisms. Once the leukocyte has arrived from the blood stream, the inflammatory process is well under way and in many cases the process is accompanied by a variety of reactions. These pathogenic effects of immune complexes are important contributors to a number of human diseases, pulpal pathology not withstanding.

Generally, tissue injury produced by immune complexes can be divided into four phases: (1) a combination of antigen and antibody and possibly complement producing complexes; (2) localization of complexes; (3) an accumulation of humoral and cellular factors; and (4) the production of tissue injury. The damage is generally a consequence of cellular interaction with the complexes and the neutrophile is the cell most clearly implicated (Cochrane, 1967).

I. Investigations Using Immunologic Assay

The application of immunologic techniques to clinical medicine has seen dramatic increases in usage in the last 10 years. The basis for these tests lies in the fact that most clinical syndromes have changes in protein concentrations. By knowing which proteins are affected and what the normal serum values are, an investigator can use the various immunological reactions to quantitate these proteins. For example, in acute rheumatic endo-

carditis, there are two main proteins of interest, C-reactive protein (correlated with active tissue break down) and antistreptolysin O. In 1959, Fukuda, developed a gel diffusion technique for quantitating C-reactive protein in serum. Likewise, an accurate gel-diffusion test is available for quantitation of antistreptolysin O. Blum, et al, in 1970, found a negative ASO test to be significant and reliable as an indicator of insignificant ASO titer.

Another area of interest for the application of immunologic assay is in neurological diseases. As described by Crawley (1969), there are four major cerebrospinal fluid protein patterns seen in neurological disease. By analyzing the ratio of cerebrospinal fluid gamma globulin to total cerebrospinal fluid protein or to the ratio of gamma globulin to albumin one can determine if abnormalities exist (Hartley, et al, 1966).

Immunological studies have also been carried out concerning dental disease states. Brill and Bronnestam (1960) carried out electrophoretic studies of gingival fluids recovered from human gingival pockets. They demonstrated gamma globulin in this fluid and hypothesized the presence of antibodies as well.

Analysis of blood serum from two hundred males, ages

seventeen to twenty-two, was preformed by Shannon and Bibson (1964). They found no correlation between total serum protein, albumin, globulin and periodontal disease.

Brandtzaeg, in 1965, employing micro-double diffusion tests developed by Ouchterlony (1949), determined that the proteins IgG, IgA, IgM, albumin and fibrinogen of gingival pocket fluid were present in proportion and in concentrations comparable to that of plasma.

Byers (1973) studied the globulin fractions IgG, IgA, IgM of resected inflamed gingival tissue. Using low level immunodiffusion plates for the assay, he found globulin concentrations to be higher in inflamed tissue when compared to normal tissue, thus indicating to him the apparent localized production of immune globulins in gingiva.

Extensive studies have also been carried out using a immunofluorescent technique to demonstrate increased globulin levels in gingival tissues. Brandtzaeg and Kraus (1965) reported dramatic differences between non-inflamed and inflamed gingiva when studied with immunofluorescence. They showed the ratio of IgA to IgG containing plasma cells greatly increased with inflammation. In 1970, Platt, Crosby, and Dalbrow used immunofluorescence to study gingivectomized tissue from patients with

periodontal disease. Fluorescing plasma cells for IgG and IgM seemed to be predominant with few recorded for IgA. The author's concluded that the host defensive mechanism provided by globulins persists through long periods of chronic inflammation.

J. Pulpal Studies

As early as 1926, investigators were interested in the fluid content of teeth. Solutions of various stains such as trypan blue and india ink, were injected subcutaneously into cats and dogs, and then the animals were sacrificed at various time intervals and their teeth sectioned for observation. In as short a time as 30 minutes, color was perceived in the dentin, which became more intense with time. It was therefore concluded that the tubules present in dentin could be considered lymph channels where by nutrient materials, oxygen, and immune bodies are transported to the living dentin and the products of its catabolism are carried away. (Fish, 1926, 1927 a and b).

Von Kreudenstein (1958) employed a method of cutting away a goodly portion of the crown of a vital tooth without exposing the dental pulp. A clear fluid was seen to appear on the surface on the dentin and was subsequently called "dentinliquor"

Haldi, et al, in 1961, found by careful drilling access to the dental pulp without cellular damage could be obtained. By inserting a capillary tube into the cavity created a clear, colorless fluid could be collected. This was subsequently assayed. It was found that the protein content of the pulpal fluid was about one-fifth that of blood plasma, whereas the glucose concentration was in equilibrium with the concentrations in the arterial blood plasma.

In a later study by Haldi and Wynn (1963), electrophoresis was done on pulp fluids obtained as described. These fluids were found to contain the same protein fractions as blood plasma, differing only in concentrations. It was suggested that the dental pulp fluid could be regarded as a capillary transudate. Further, it was their contention that globulin escapes more readily from capillaries than does albumin. Likewise, proteins in the inflamed pulp show a much higher concentration than in the non-inflamed pulp, (Aono et al, 1964).

Meanwhile, in Japan, further pulp plasma protein studies were being carried out. As described by Honjo, 1968, Sato and Yokomizo did electrophoresis on dental pulp homogenate. They found albumin, alpha, beta, and gamma globulin fractions in the supernatant. Honjo et al,

(1968, 1970) employed fluorescent antibody techniques to detect homologous plasma proteins in human pulp tissue. Using both inflamed and non-inflamed samples of dental pulp tissue, albumin, gamma globulin and fibrinogen were localized. In the inflamed specimens, a wider spread of stain was found depending on the amount of inflammation. Further, most of the immunoglobulin containing cells possessed IgG and very little IgA or IgM. This led them to believe that a local antibody production may occur in the dental pulp.

It has been reported that if tissue components are damaged and/or modified by trauma, drugs or infection, they could be rendered antigenically active to the host, (Kaplan, 1958; Weigle, 1964). Moreover, as cited by Nishida (1971), Kimura reported a systemic allergic response was induced as a result of sensitization with this type of modified tissue antigen, if retained over long periods of time within the tissue. This possibility has also been suggested for chronic dental foci (Dietz, 1952). Formaldehyde has been widely used to inactivate bacteria, viruses, and toxins while retaining their antigenicity. In addition, it has been claimed by some investigators (Jacobs & Sommers, 1939) that the treatment of tissue components with formaldehyde could render them antigenically

active to the host. In view of the possibility that tissues of the dental pulp treated with formalin could acquire antigenicity, with sensitization of the host, Nishida, et al, (1971) investigated antibody production to formalin treated rabbit dental pulp extracts and he found homologous antibodies in the IgG and IgM class by ultracentrifugation.

The immune response has been proposed both as a mechanism of protection and as a mediator of injury. Immune globulins have been described in normal and inflamed human pupal tissue, but to this date, no accurate quantitation of their levels has been made. It is the design of this paper to compare the immunocompetance of inflamed and non-inflamed human pulp, by quantitation of IgG, IgA, IgM, C'3 factor of complement and albumin.

CHAPTER III

MATERIALS AND METHODS

Human dental pulps were obtained from teeth extracted in the oral surgery clinic at Loyola University Dental School. Teeth were catagorized as to inflamed and non-inflamed by the following criterion: non-inflamed teeth were those teeth with no caries or bone loss due to periodontial disease and assumed to be non-pathologic; inflamed teeth had carious exposures of apparent long term duration due to extensive destruction of tooth structure. Due to the small amount of tissue obtained from each tooth all pulps in each category were pooled.

Eighteen subjects represented the non-inflamed group ranging in age from thirteen to forty-eight years with a mean age of 24.3 years including seven males and eleven females, a total of thirty-three pulp samples were obtained from this group (Fig. 5). Fourteen subjects participated in the inflamed group ranging in age from nineteen to sixty-eight years with a mean age of 38.9 years, including 6 males and 8 females yielding thirty-one pulpal samples (Fig. 6). As well as extracting teeth from

the above patients, a finger prick blood sample was obtained for each patient and the serum pooled. Each patient contributed approximately 1/10 ml. of serum per tooth used in the study. All of the patients used for this experiment were in good general health and were not presently taking any medication. The extraction procedures were performed under local anesthetic employing regional blocks.

Immediately after extraction each tooth was cracked by percussion with a mallet and the pulp was removed intact (see illustrations in appendix). The pulpal tissue was examined macroscopically to determine the quality of the tissue. The inflamed samples were found to have localized abcess formation with some areas of intact pulp. The non-inflamed specimens were totally intact with no apparent pathology. All teeth which showed purulent pulps were not included in this study. The pulpal samples were promptly placed into a labeled serum bottle containing 1 ml. normal saline (0.85%) maintained in a crushed ice bath during the collection period. At the end of the collecting period the bottle was tightly stoppered with rubber stoppers and metal caps. The bottles were then placed into a Revco ultra low freezer at minus eighty-five degrees centigrade.

The blood sample was collected in hematocrit tubes

and centrifuged for three minutes in a International micro capillary centrifuge. The serum fraction was decanted and stored in a small pyrex specimen bottle and maintained in a crushed ice bath during the collection period. At the end of the collecting period the serum samples were frozen and stored as above.

After pulp samples were collected, saline extraction of the globulins was carried out. Sufficient diluent as determined in a pilot study, was added to the samples. After dilution, the tissue was placed in a ground glass tissue homogenizer and maintained in a crushed ice bath during ten minutes of grinding. The resulting homogenate was placed in fifteen milliliter glass centrifuge tubes and centrifuged in a Beckman JA 20 centrifuge for thirty minutes at 20,000 R.P.M.'s at four degrees centigrade. The final volume of supernatant fluid was recorded. The straw colored supernatant fluid was pipetted off in three milliliter aliquots and placed in a 5-milliliter screw-cap Vac Vial. The liquid sample was then shell frozen in dry ice acetone mixture and placed on a lyophilizing manifold and maintained overnight until dry. The resulting powder samples were removed from the manifold and held in a Revco freezer until needed. This procedure was used for the pilot study samples, the inflamed pulp samples, and the

non-inflamed samples.

The tissue extracts were assayed for immunoglobulins IgG, IgA, IgM, C'3 fraction of complement and albumin using regular and low-level immuno-plates.¹ Immuno-plate immunodiffusion plates are used to quantitate a variety of proteins in serum and other body fluids. These proteins are of such a complex chemical nature that they have been difficult to identify or to differentiate by conventional chemical and electrophoretic techniques. By usual electrophoretic methods, serum proteins are classified into groups designated as albumin, alpha₁ globulins, alpha₂ globulins, beta globulins and gamma globulins (see Fig. 1, page 4). A number of protein fractions present in serum are not readily distinguishable by electrophoresis, usually due to overlapping mobilities. Immunodiffusion techniques are specific for identification and quantitation of those proteins for which specific antibodies have been developed. Proteins which presently have an immunological quantitation system include IgA, IgM, IgG, IgD, complement C'3, alpha₂ macroglobulin and albumin. The immuno-plate utilizes a technique described by Fahey and McKelvey (1965) where an agar plate is prepared in incorporating antibody throughout the agar. Six or twelve identically sized and

¹ Hyland, Division Travenol Laboratories, Costa Mesa California.

positioned wells are prepared in the agar to receive the sample. The test sample is placed into the well and, on diffusion into the agar, forms a ring of antigen-antibody precipitate around the well. The diameter of the precipitate ring reflects the concentration of the antigen. Further, it was these author's opinion that this test could be used to quantitate proteins at a low concentration of 3 mg% with a maximum of $\pm 10\%$ error.

One-half hour prior to assay the frozen lyophilized samples were removed from the freezer and allowed to thaw at room temperature. Serial dilutions of standards were supplied by Hyland Laboratories in the following concentrations: IgG (320mg/100ml, 1000mg/100ml, 2000mg/100ml), IgA (67mg/100ml, 205mg/100ml, 430mg/100ml), IgM (40mg/100ml, 120mg/100ml, 260mg/100ml), C'3 complement (52mg/100ml, 160mg/100ml, 321mg/100ml), Albumin (13mg/100ml, 53mg/100ml, 150mg/100ml, 330mg/100ml), low level IgG (3mg/100ml, 9.4mg/100ml, 43mg/100ml, 125.5mg/100ml) low level IgA (4.5mg/100ml, 10.5mg/100ml, 21mg/100ml, 43mg/100ml), and low level IgM (3.75mg/100ml, 12.1mg/100ml, 24mg/100ml, 49mg/100ml). These samples were placed on the immuno-plates in triplicate and incubated. After thawing the tissue samples were reconstituted with one milliliter of distilled, deionized water. The tissue samples and serum samples

were then serially diluted with saline as follows: 1:2, 1:4, 1:8, 1:16, 1:32. These dilutions and the concentrated samples were run in duplicate for all the tests mentioned previously. The plates were incubated at twenty-two degrees centigrade (room temperature) in 100% humidity. IgG, IgA, IgM C'3 fraction of complement, albumin, low level IgA, and low level IgM were incubated for 16 hours and read immediately. The low level IgG plate was incubated for four hours and read. The precipitant ring diameters (Fig. 9) were measured with a caliper and millimeter measure to 0.5mm., the plates being illuminated by the immunoilluminator.² The mean diameters from the duplicate and triplicate runs were used for final determinations. Protein levels were plotted on the Y-axis of semi log graph paper. Using the known serial dilutions, a standard curve for each protein fraction was drawn and the unknown protein concentrations were read from the standard curve (Figs. 10-16).

²Hyland Laboratories, Costa Mesa, California.

CHAPTER IV

RESULTS

The concentrations of immunoglobulin IgG, IgA, IgM, C'3 factor of complement, and albumin were determined for the pooled samples of non-inflamed pulp tissue, inflamed pulp tissue, serum samples from patients in the inflamed group, and serum samples for patients in the non-inflamed group.

Comparing the serum samples of the inflamed group to the non-inflamed group the following results were noted: 1) mean concentration of IgG is comparable (1033 mg % to 988 mg %); 2) the mean concentration of IgA seems to be approximately 2:1 (355 mg % to 192 mg %); 3) the mean concentration of IgM is also comparable (102 mg % to 167 mg %); the mean concentrations of albumin are comparable (3040 mg % to 3760 mg %), the mean concentration of C'3 complement is approximately 2:1 (211.6 mg % to 129.3 mg %). The mean protein levels for normal human serum as supplied by Hyland Laboratories are as follows: IgG 1200 mg %; IgA 288 mg %; IgM 145 mg %; Albumin 4830 mg %; C'3 145 mg % (See Table 3).

TABLE III

Concentrations of Serum Proteins

	Serum (mg per 100ml)				C'3 Complement
	IgG	IgA	IgM	Albumin	
Inflamed group	1033	355	102	3040	211.6
Non-Inflamed group	988	192	167	3760	129.3
	Pulp (mg per 100 ml)				C'3 Complement
	IgG	IgA	IgM	Albumin	
Inflamed group	97.2	7.75	0	96	26
Non-Inflamed group	47	4	0	111.3	23
	Normal Human Serum (mg per 100 ml) ³				C'3 Complement
	IgG	IgA	IgM	Albumin	
	1200±319	288±121	145±100	4830±900	145±22

³ Values supplied by Hyland Laboratories, Round Lake, Illinois
variability of ±10% as per Fahey (1965).

Comparing pulp concentrations, inflamed versus non-inflamed, yielded these results: 1) the mean concentration of IgG was approximately 2:1 (97.2 mg % to 47 mg %); 2) the mean concentration of IgA also appeared to be approximately 2:1 (7.75 mg % to 4 mg %); 3) the concentration of IgM was not recordable; 4) the mean concentration of albumin was approximately the same (96 mg % to 111.3 mg %); 5) the mean concentration of C'3 complement is about the same (26 mg % to 23 mg %) (See Table 4).

A different view of the results can be achieved by comparing serum and pulp fluid concentration. By using the ratio of pulp protein to serum protein concentration the following figures are available: 1) the ratio of albumin in both inflamed and non-inflamed are identical (0.03); 2) the ratio for IgG is approximately 2:1 inflamed versus non-inflamed (0.094 to 0.048); 3) values for IgM were not available for pulpal samples; 4) IgA ratios were almost the same (0.022 to 0.021); 5) the ratio for C'3 complement are almost 1:2 inflamed versus non-inflamed (0.12 to 0.21) (See Table 4).

Another method for analyzing the data was employed by comparing the ratio of protein concentration to albumin concentration. This analysis shows any increase or decrease

TABLE IV

Comparison of Serum and Pulp Fluid Concentrations

Ratio of Pulp Protein to Serum Protein Concentrations

	Albumin	IgG	IgA	C'3 Complement
Inflamed group	0.03	0.094	0.022	0.12
Non-Inflamed group	0.03	0.048	0.021	0.21

as compared to albumin which is the marker protein in this study. It eliminates any increase in protein concentration due to serum volume increase. Comparison of the serum

samples first, the ratio of inflamed versus non-inflamed:

1) Albumin has a ratio of 1 to 1; 2) IgG has a ratio of 0.34 to 0.26; 3) IgA has a ratio of 0.12 to 0.05; 4) IgM has a ratio of 0.03 to 0.04; 5) complement has a ratio of 0.07 to 0.03 (See Table 5). Examination of pulpal samples, inflamed versus non-inflamed, gives these results; 1) albumin has a ratio of 1 to 1; 2) IgG has a ratio of 1.01 to .42; 3) IgA has a ratio of 0.08 to 0.036; 4) IgM has no results; 5) complement has a ratio of 0.27 to 0.21 (See Table 5).

Graphic interpretation of these results can be obtained by referring to figure 3 and 4. Standard curves plotted for calculation of unknown protein concentrations are found in the appendix.

TABLE V

Comparison of Immunoglobulins and Other Protein Components

Ratio of Protein Component to Albumin

	Albumin	IgG	IgA	IgM	Complement
<u>Serum</u>					
Inflamed group	1	0.34	0.12	0.03	0.07
Non-Inflamed group	1	0.26	0.05	.04	0.03
<u>Pulp</u>					
Inflamed group	1	1.01	.08	0	0.27
Non-Inflamed group	1	.42	.036	0	0.21

Figure 3
Concentration of Protein Constituents
Non-Inflamed Subjects

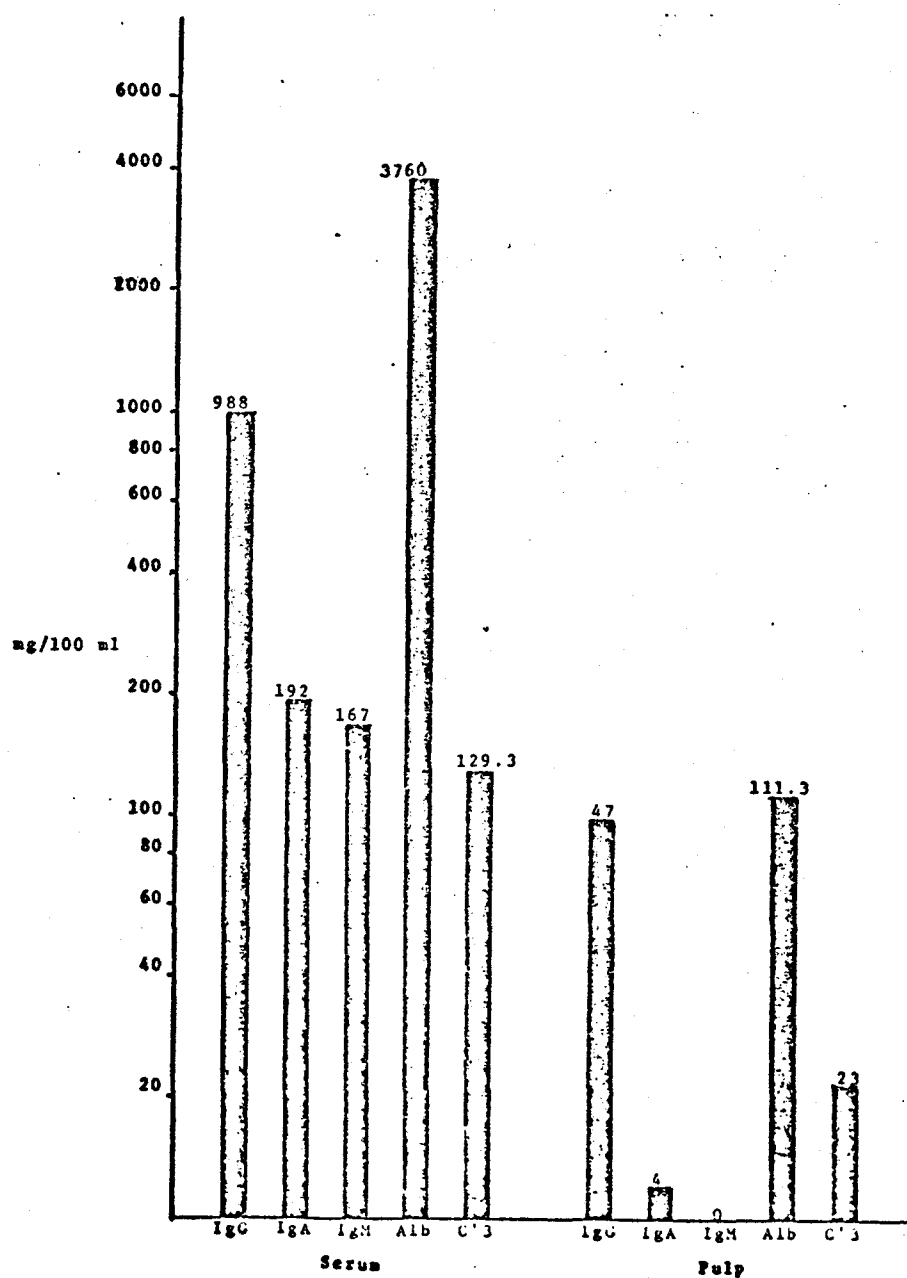
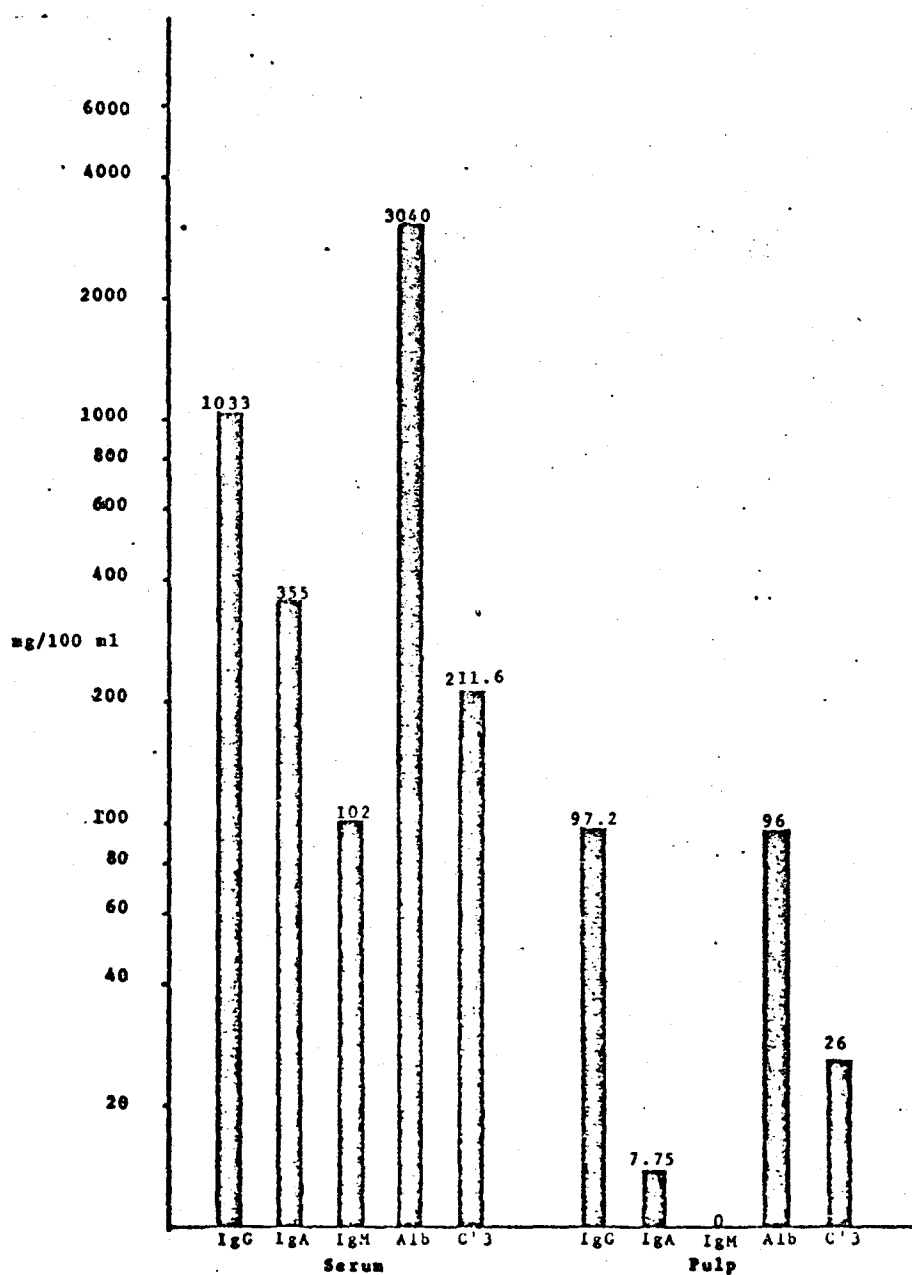


Figure 4
Concentrations of Protein Constituents
Inflamed Subjects



CHAPTER V

DISCUSSION

This study has shown that there is a marked increase in immunoglobulins IgG, IgA, and an apparent decrease in C'3 fraction of complement in inflamed pulpal tissue when compared to non-inflamed pulp tissue. The protein components of the pulp tissue were saline extracted and assayed with immunodiffusion plates. Examining results of other tissue assay studies employing various immunologic techniques, one finds demonstration of increased levels of immunoglobulins in inflamed tissue versus non-inflamed tissue. However, quantitation of these globulins was never in agreement (Byers; Brandtzaeg and Kraus; Platt, et al; Aono; Honjo).

Utilizing the immunodiffusion technique for assaying tissue samples for serum protein quantitation offers a number of advantages. The antibody incorporated into each plate was specific for the protein to be evaluated. The plates demonstrated sufficient accuracy for routine clinical usage, furthermore, results were readily reproducible (Fahey, 1965). The immuno-plates offered a wide working

range when the low level plates were used in conjunction with regular plates; it was possible to detect protein concentrations as low as 3 mg percent. This quantitation technique appears to be superior to techniques employed by other authors in previous tissue assay studies. Electrophoresis studies while accurate in separating protein fractions of various electrophoretic mobilities, lack the ability to quantitate specific globulins or protein fractions. Only a mono-specific antibody-antigen reaction can accomplish this. Likewise, immunofluorescent are not always mono-specific which complicates accurate quantitation. For example, disaggregated collagen which is often found in inflamed tissue will give a positive fluorescence for IgA and IgM.

After examination of the mean concentrations of serum proteins in pulpal and serum samples certain trends were noted with regard to the concentration of IgG, IgA, and the C'3 fraction of complement. It appeared from this table that pulpal concentrations of IgG and IgA were approximately 2:1 when comparing inflamed to non-inflamed (See Table 3). However, to fully utilize the serum marker protein, albumin, it was necessary to compare these figures differently. In table 4 a comparison was made between serum and pulp fluid concentrations. When looking at this

table, one can compare differences after equalizing any increase or decrease due to blood serum concentration. The most interesting finding here is the relative decrease in C'3 complement concentration when comparing inflamed to non-inflamed samples. As can be noted by examining tables 3 or 5 this difference does not appear. This is due to the fact that blood serum concentrations were not equalized in those figures. Further, table 3 and table 5 would lead one to believe complement should have been higher in the inflamed group as indicated by the level in the inflamed serum. However, the figures for pulps were similar due to the fact it was consumed in the inflamed condition. This decreased level of C'3 fraction of complement in inflamed tissues is confirmed by the experimentation reported by Cochrane and Muller-Eberhard, in 1967.

The marked increase in immunoglobulin IgG and IgA in the inflamed pulp samples when compared to non-inflamed pulps is probably due to the antigenic stimulus of micro-organisms in the pulp as well as the cellular breakdown products from the preceeding or ongoing inflammatory responses. Due to the unique environment of the dental pulp, the antigens and the toxic bacterial products are not readily accessible for removal by cleansing nor are they readily subject to dilution by the fluids of the oral cavity.

Prolonged exposure to microorganisms may eventually breakdown the first lines of defense provided by the enamel and the dentin surrounding the dental pulp subsequently allowing the ingress of antigenic substances to the connective tissues of the pulp. This could result in the mobilization of the immune system at the local and possibly the systemic level. Immunoglobulins specific for oral microorganisms have been demonstrated in serum antibody investigations (Mergenhagen, et al, 1965). The increased level of immunoglobulins exhibited in this study could be due in part to a localized synthesis by local differentiating immunocompetent cells or a production of immunoglobulins by cells of the plasmacyte series which have migrated to the area. This could explain the presence of the large numbers of lymphocytes and plasma cells found when histologic sections of inflamed pulps are examined. This factor is usually not discussed when examining these microscopic sections. Most often it is noted that acute inflammation shows a predominance of PMN's and chronic inflammation shows an increased number of round cells. The immunocompetent cells of the dental pulp appear to synthesize antibodies in response to challenges from the antigenic capacities of microorganisms. However, these immunocompetent cells must continually synthesize immunoglobulins since the half-life of IgG is approximately 23 days and IgM and IgA

are much shorter (Barth 1964).

The lack of findings for IgM in the pulp specimens can be accounted for with these arguments. In as much as all the inflamed pulp samples came from cariously exposed teeth one would expect to find long standing inflammation. The production of IgM is usually the first response to a antigenic challenge followed closely by that of IgG. The level of IgM peaks within a few days and further production seems to be inhibited by feedback mechanisms connected to the production levels of IgG. Also, the large pentameric molecule of IgM makes it susceptible to fractionization by the freeze-thaw method employed in this experiment. This could render the molecule antigenically inactive to the specific antibody on the immunodiffusion plates.

There are some interesting clinical significances which can be deduced from this study. It is apparent from the increased levels of immunoglobulins that the pulp has the tissues and cells available to mobilize the immune response, whether the antigenic stimulus comes from micro-organisms in the oral cavity or from cellular breakdown products. As was pointed out earlier, antibody titers do exist to normal oral flora. This means the first line of defense after the hard structures of the tooth are breached could be the circulating antibodies. Subsequently, an

arthus reaction could occur pulpally with circulating antibody complexing the foreign antigens and beginning the humoral as well as the cell mediated immune response, possibly leading to further tissue damage. It appears, therefore, that pulpal integrity could be destroyed by the loss of physiological function due to immunologically mediated tissue destruction as by acutal microbial invasion. A traumatic incident could also effect the pulp by means of immunologically mediated tissue destruction. Locally damaged portions of the pulp due to a deep cavity preparation, for example, could cause inflammation and subsequent tissue breakdown as described eariler. If this inflammation and tissue destruction is not contained the entire pulp could become involved. This would allow the degradated products to penetrate the periapical tissue and possibly cause a lesion formation. A similar sequence of events could occur due to the presence of necrotic tissue in contact with viable tissue, dead tracts in dentin, necrotizing agents applied to dentin, or pulp stumps after pulpotomy are such examples. Kaplan (1958) and Dietz (1952) described that alteration of antigenic activity is possible when tissue components are damaged by long standing inflammation, trauma, drugs, or infection.

The pooling technique used in this study has weaknesses,

unfortunately the inherent size of the dental pulp makes individual samples impractical. The criticism lies in the fact that pooling samples eliminates the possibility of reporting ranges in protein concentration and the fact that an extremely high or low sample will go undetected and could effect the mean substantially.

The saline extraction of the examined for proteins could also lead to possibilities of error. Tissue fixed antibodies may have remained in the cellular debris after centrifugation which could mean the actual concentrations of antibodies may be even higher.

It was necessary to assume advanced inflammation existed in the cariously exposed teeth as in sufficient material remained for each sample to make a histologic confirmation.

Byer's studies (1973) did not include a marker protein such as albumin. Because of this he was unable to draw conclusions relative to the immunoglobulin concentration due to serum in the tissues. Both this study and Byer's work demonstrated increased globulin levels in their respective inflamed tissues. However, quantitative comparisons cannot be made due to differences in methodology.

Further studies are necessary to more fully correlate the clinical observations with the histologic findings,

and with the quantitative measure of the immunoglobulin and complement levels in the inflamed dental pulp. Work must also be directed to more clearly relate developing modern concepts of inflammation as they pertain to the dental pulp. Future studies should include electronmicroscopy of the cellular components of the dental pulp during inflammation, studies of the antibody levels relating to specific antigens within the pulp, and possibly the use of immunofluorescent techniques. These studies would provide further insights into the mechanisms of response that pulpal tissue undergoes.

CHAPTER VI

SUMMARY AND CONCLUSION

Human dental pulps were obtained from teeth extracted at the oral surgery clinic at Loyola University Dental School, for the purpose of assaying immunoglobulins IgG, IgA, IgM, C'3 fraction of complement and albumin. Teeth were catagorized as inflamed if they had carious exposures and non-inflamed if they had no caries or any bone loss due to periodontal disease.

Eighteen subjects represented the non-inflamed group ranging in age from thirteen to forty-eight years, including seven males and eleven females. Fourteen subjects represented the inflamed group ranging in age from nineteen to sixty-eight years, including six males and eight females. Each patient also contributed approximately 1/10 ml of serum per tooth used in this study. Tissue specimens were prepared and immunoglobulins, C'3 complement and albumin levels determined by immunodiffusion assay.

The mean concentrations of IgG and IgA were greater in the inflamed samples than in the non-inflamed samples. This indicates the apparent existance of an immune response in inflamed pulpal tissue with possibly localized synthesis

of immunoglobulins.

The mean concentration of C'3 fraction of complement was shown to be markedly decreased in the inflamed sample versus the non-inflamed sample. This indicates that the inflammatory response existing in the inflamed group is utilizing C'3 complement. IgM was not demonstrated in the pulpal samples, indicating that IgM is not present in human pulps in sufficient quantities to be detectable by the assay method employed in this study.

The following conclusions can be made from this study:

- 1) IgG, IgA, C'3 fraction of complement, and albumin are present in both inflamed and non-inflamed human dental pulp tissue.
- 2) The mean concentration of IgG and IgA seem to be substantially higher in inflamed pulps when compared to non-inflamed pulps.
- 3) The mean concentration of C'3 fraction of complement appears to be lower in inflamed pulp tissue when serum levels were considered and compared to non-inflamed pulp tissue.
- 4) IgM cannot be demonstrated in the human dental pulp with this assay technique.
- 5) A local immune response appears to be occurring in inflamed dental pulps.

- 6) The saline extraction technique and immunodiffusion assay method used in this study are useful tools in quantitating the immunocompetence of the dental pulp.

CHAPTER VII

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CHAPTER VIII

APPENDIX

Figure #5

Sample Distribution

Non-Inflamed Group

	Age	Teeth Selected	Number of Blood Samples
1. Male	14	4 Premolars	4
2. Male	26	1 Molar	1
3. Female	25	1 Molar	1
4. Male	25	4 Premolars	4
5. Male	23	1 Molar	1
6. Male	17	1 Premolar	1
7. Female	31	1 Molar	1
8. Female	23	1 Molar	1
9. Female	17	2 Molars	2
10. Male	22	2 Molars	2
11. Female	24	1 Molar	1
12. Female	24	1 Molar	1
13. Female	18	1 Premolar	1
14. Female	48	2 Premolars	2
15. Female	25	1 Molar	1
16. Male	27	2 Premolars	2
17. Female	13	2 Premolars	2
18. Female	36	<u>4</u> Anteriors; 1 Premolar	4

Total 33

Figure #6

Sample Distribution

Inflamed Group

	Age	Teeth Selected	Number of Blood Samples
1. Male	19	1 Molar	1
2. Female	25	2 Molars	2
3. Male	31	1 Molar	1
4. Female	35	2 Molars	2
5. Female	68	2 Premolars	2
6. Female	23	2 Premolars; 3 Molars	5
7. Female	22	1 Molar	1
8. Female	50	2 Molars	2
9. Female	49	1 Premolar; 2 Molars	2
10. Female	43	1 Molar	1
11. Male	65	4 Premolars	4
12. Male	43	1 Cuspid; 1 Molar	2
13. Male	45	1 Premolar; 2 Molars	3
14. Male	27	<u>2</u> Molars	2

Total 31



Figure 7: Fracture Tooth Exposing
Pulpal Contents

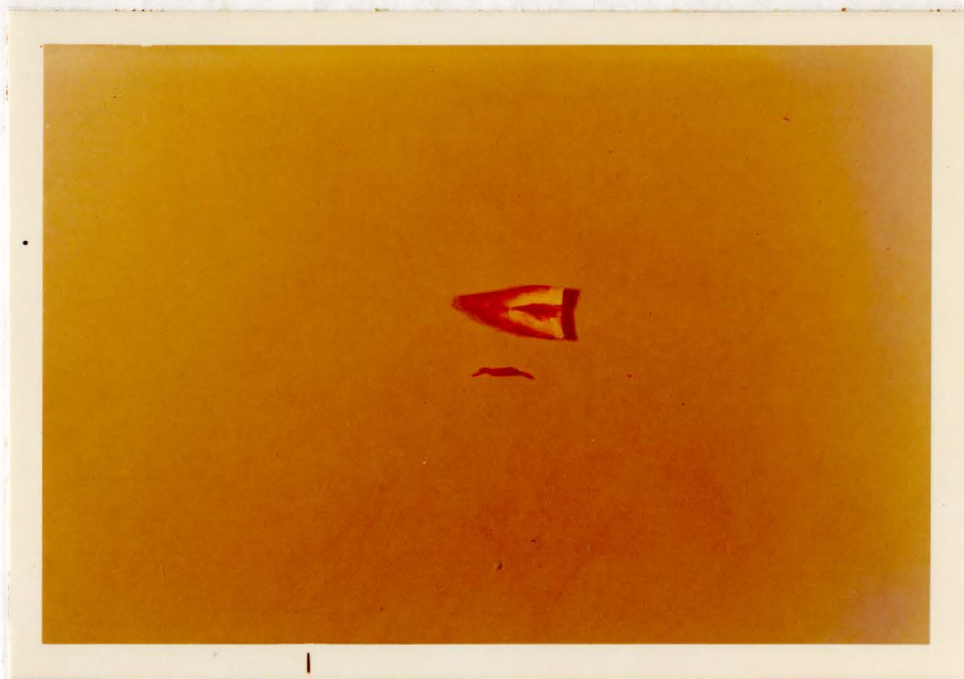


Figure 8: Pulpal Contents Extripated
on Isotonic Diffusion Plates

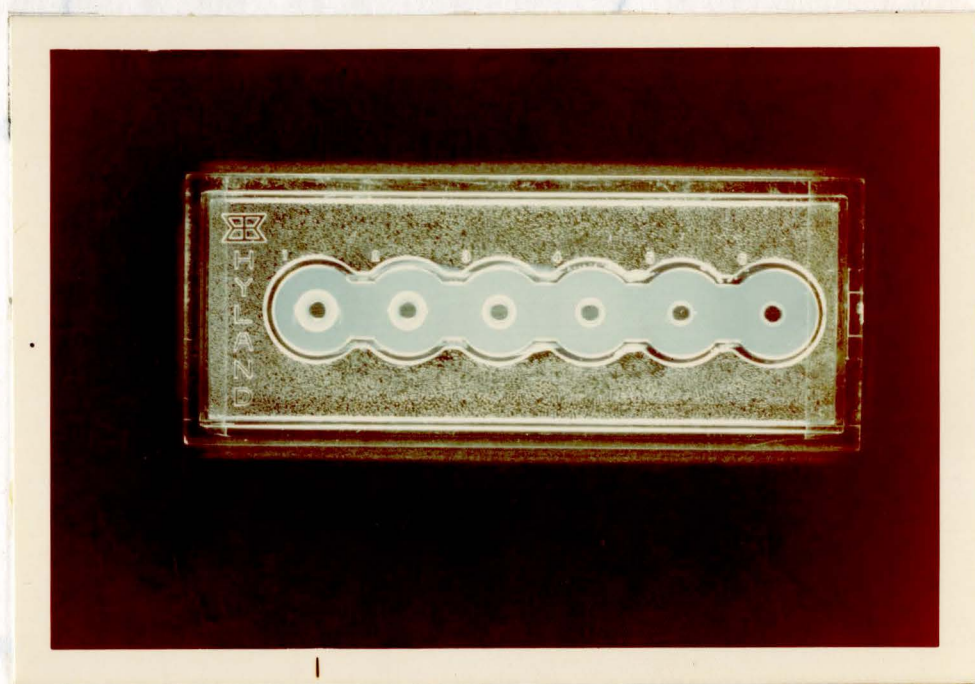


Figure 9: Demonstration of Precipitin Rings on Immunodiffusion Plates

Figure 10
Standard Curve for Low Level IgG

71

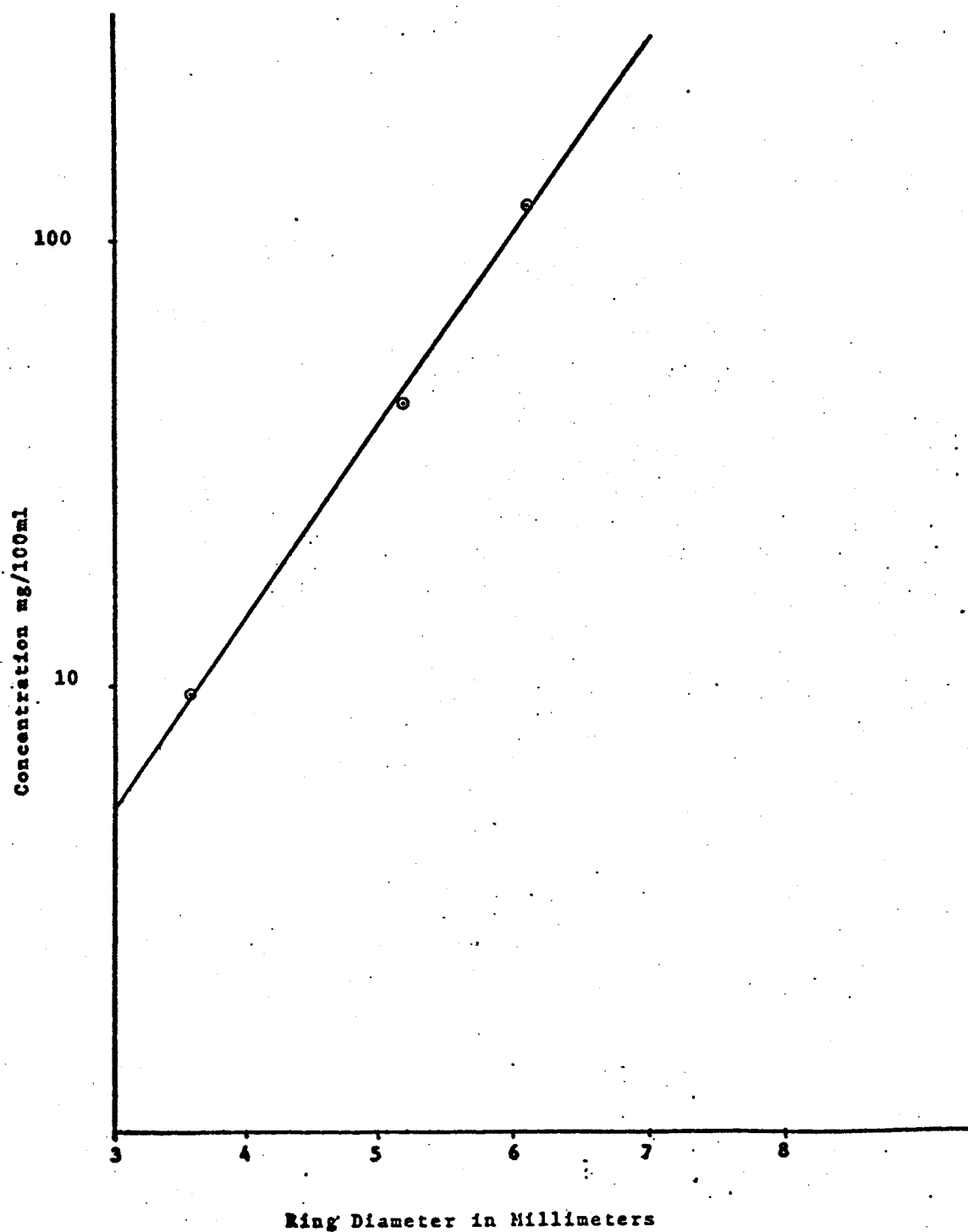


Figure 11
Standard Curve for Low Level IgA

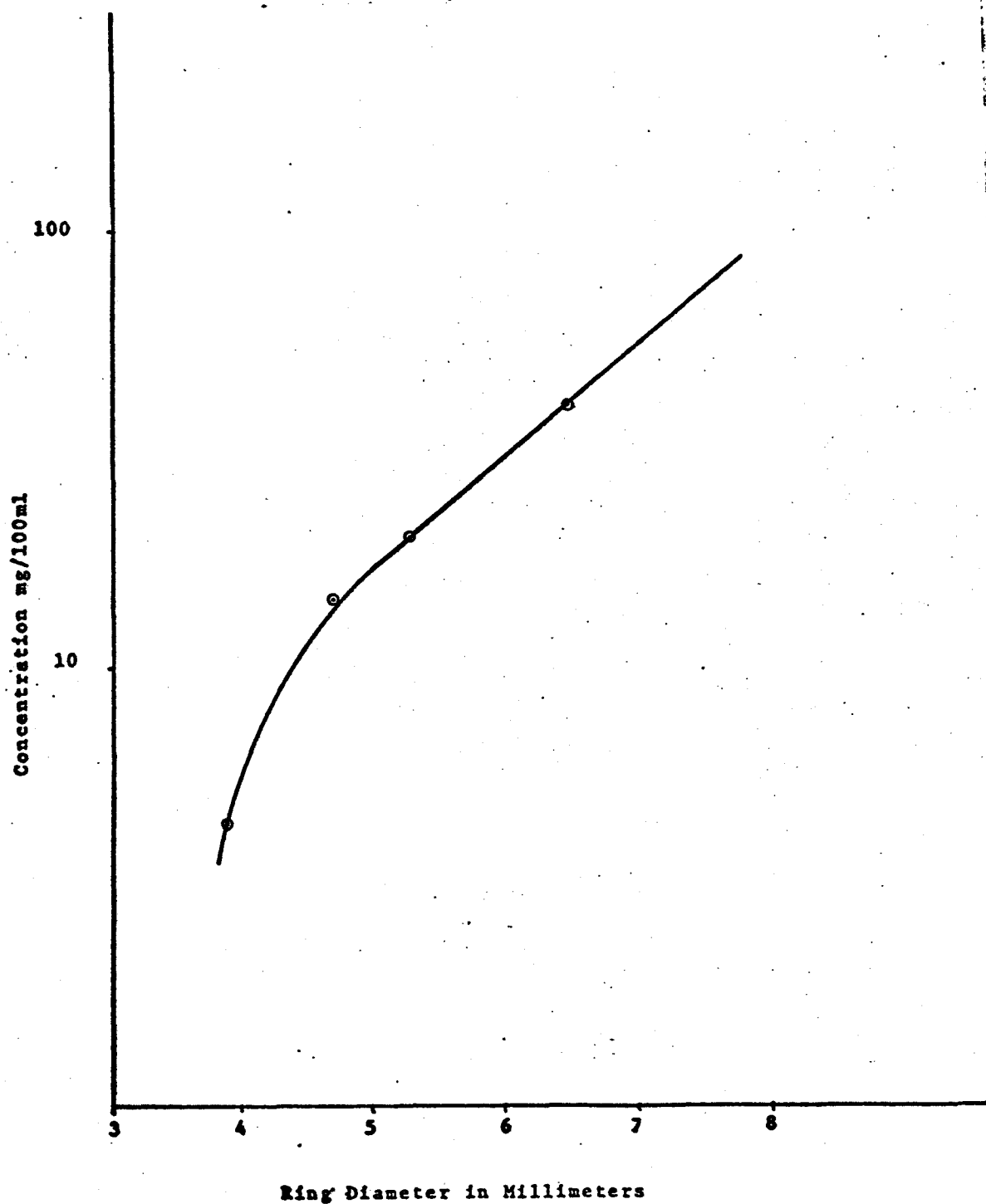


Figure 12
Standard Curve for IgG

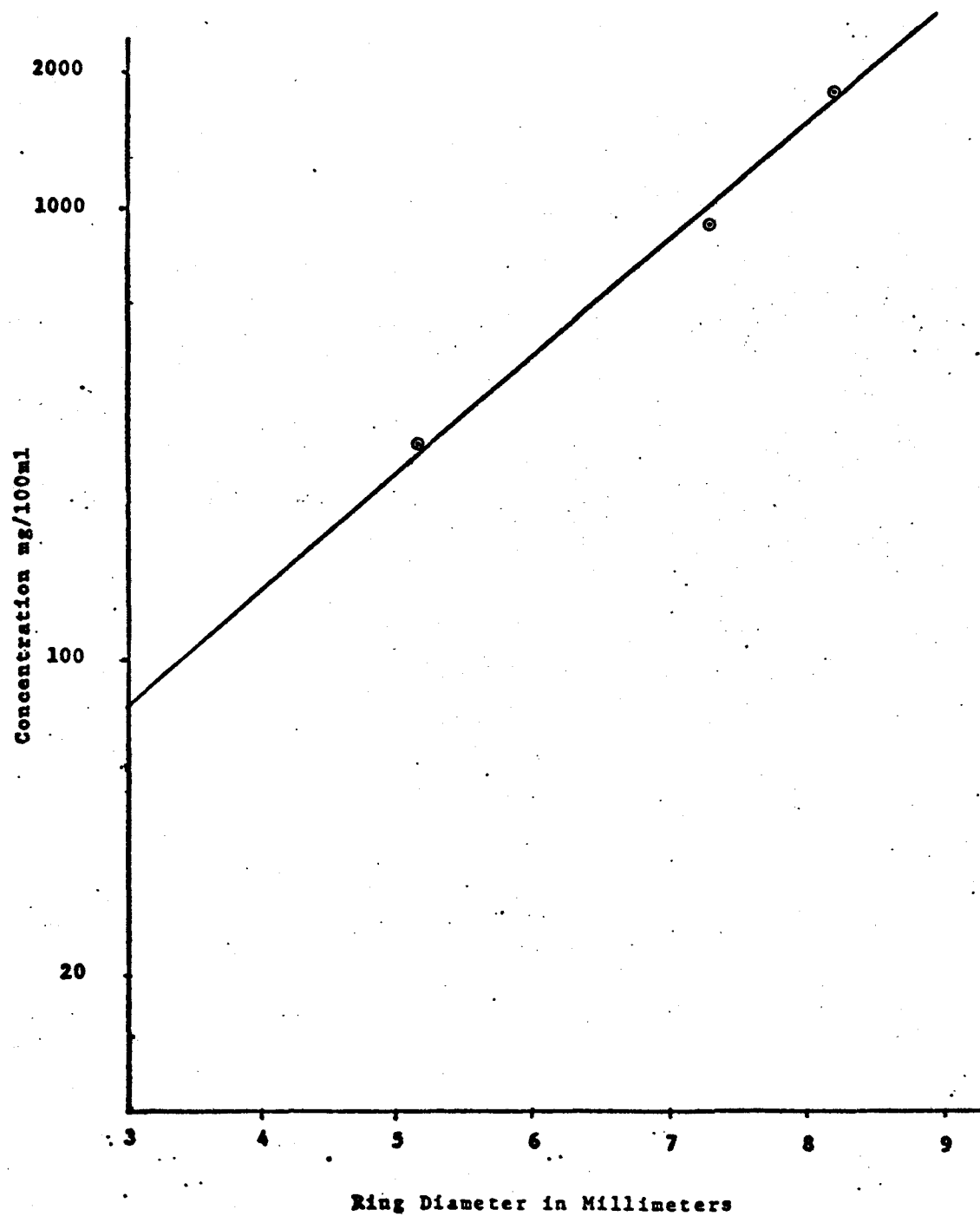


Figure 13
Standard Curve for IgA

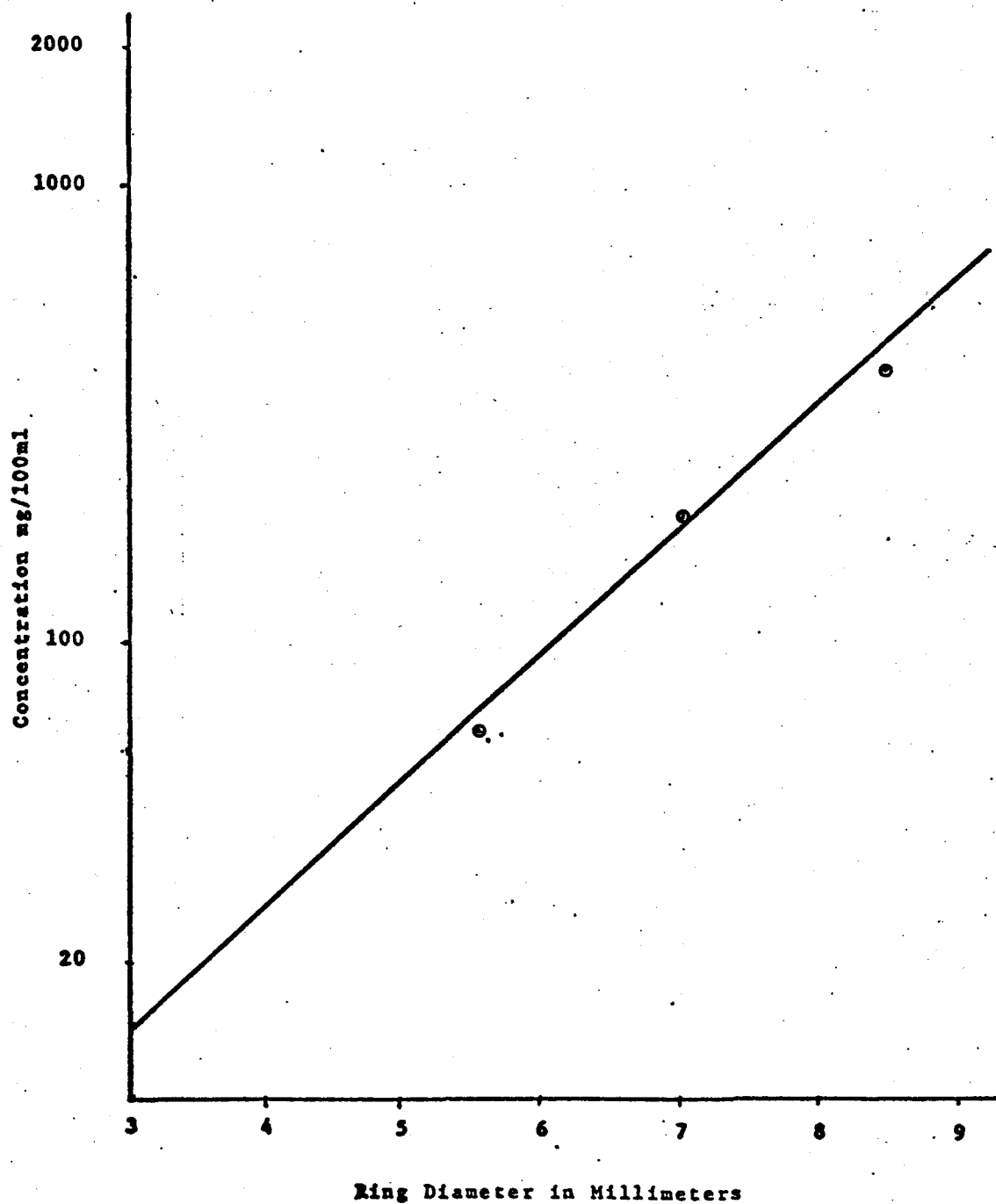


Figure 14
Standard Curve for IgM

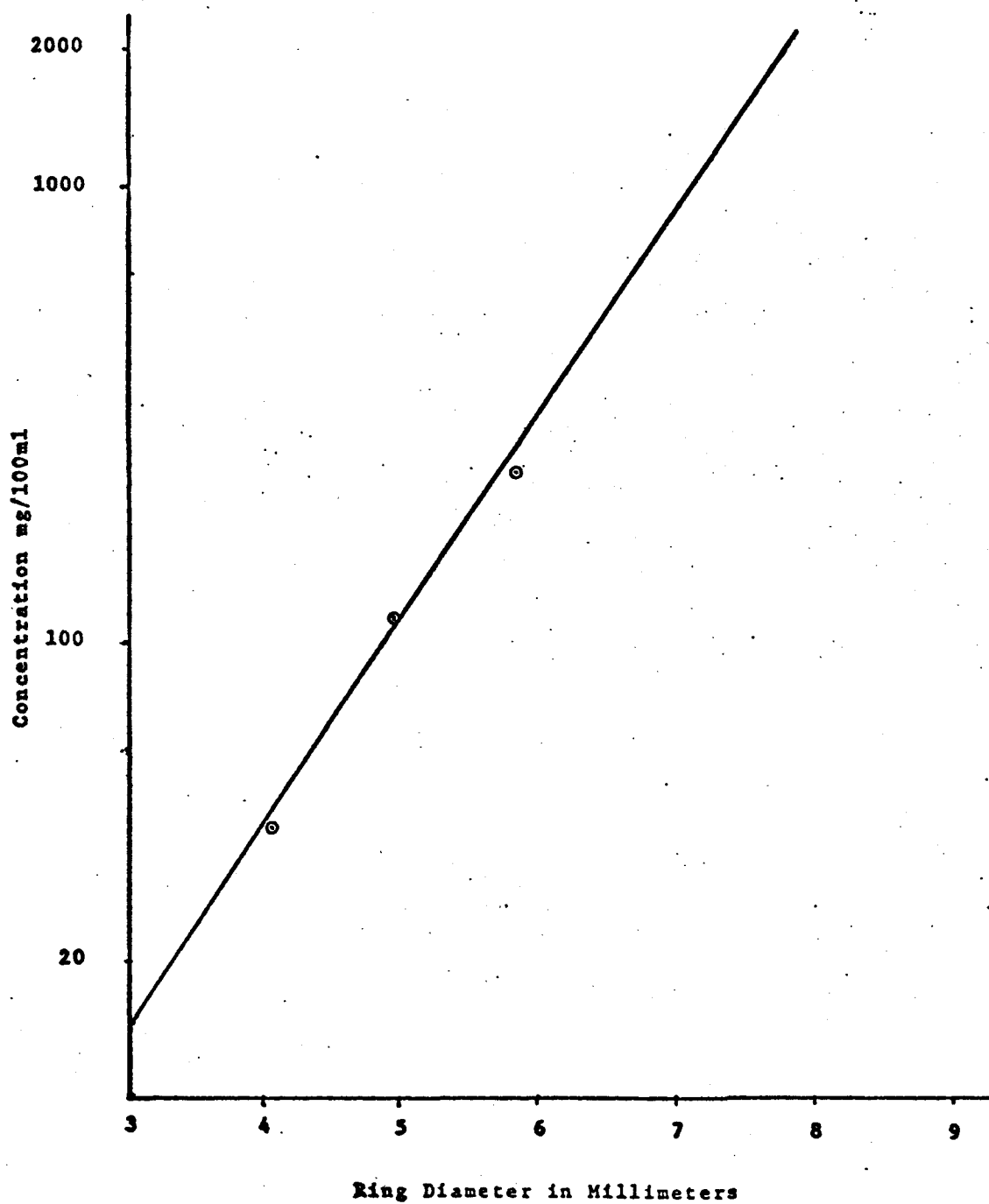


Figure 15
Standard Curve for Albumin

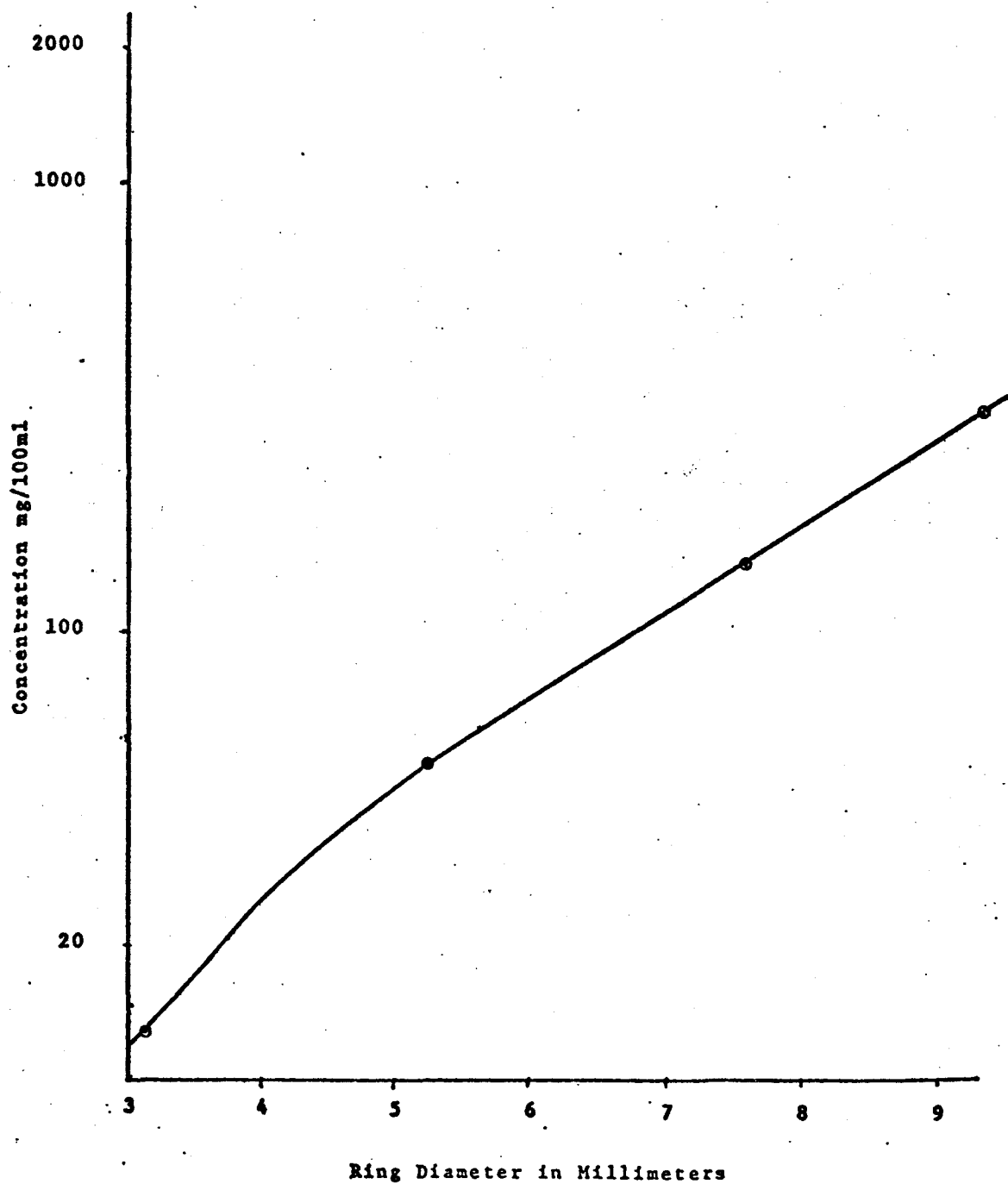


Figure 16
Standard Curve for C'3 Complement

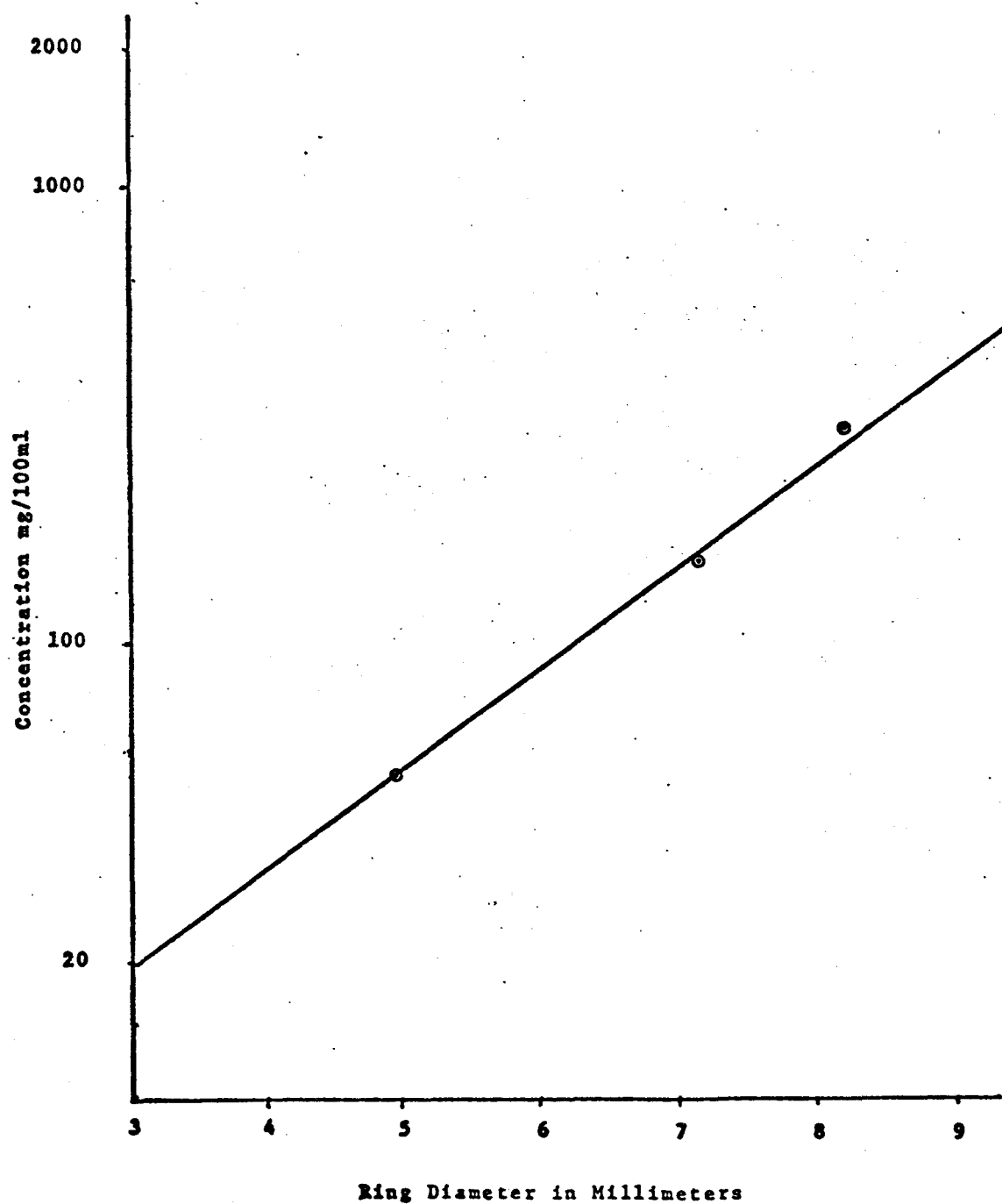


TABLE VI

Commercial Lot Numbers For
Immunodiffusion Plates ³

Low Level IgA	7092K002A1
Low Level IgG	7072G003B1
Regular IgA	7012G005A
Regular IgG	7005G008F1
Regular IgM	7022G006F1
Human Albumin	7102G001C1
Human C'3 Complement	7052G005B1

³Hyland Laboratories, Costa Mesa, California.

APPROVAL SHEET

The thesis submitted by Dr. Marvin L. Speer has been read and approved by three members of the Graduate School faculty.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with references to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 17, 1974
Date

Joh V. Madonia D.D.S. Ph.D.
Singnature of Advisor